

Towards a better understanding of the mechanisms of coral bleaching

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Coral reefs are among the richest and the most diverse biological ecosystems on Earth. The persistence, the resilience and the ecological success of reef-building corals in tropical oligotrophic waters relies on the symbiosis between cnidarians and photosynthetic dinoflagellates of the genus *Symbiodinium*. This association allows the transfer of highly energetic compounds and an efficient recycling of growth-limiting nutrients (CO₂, nitrogen and phosphorus) in the oligotrophic environment that are tropical seas (Yellowlees et al. 2008). During last decades coral reefs had to face several large-scale bleaching events compromising their survival (Oliver et al. 2009). This phenomenon linked to anthropogenic climate change and El-Niño events is triggered by warming ocean temperatures in combination with high levels of solar irradiance (UV and/or visible radiations; Lesser (2011)). In these conditions, the mutualistic relationship between host cells and their intracellular symbiotic *Symbiodinium* sp. becomes unstable and results in the loss of the algae by the host organism (Weis 2008).

The causes of coral bleaching

It has been shown at the end of the 80's that elevated solar radiation and temperature impair the photosynthetic activity and induce an oxidative stress in symbiotic cnidarians and in cultured *Symbiodinium* (Lesser et al. 1990). Since then a great deal of evidence has been collected, progressively lifting the veil on the cellular mechanisms leading to the disruption of the symbiosis. It has generally been found that the initial step of bleaching is very likely linked to photosynthesis in *Symbiodinium*. Indeed, thermal and light stress were reported to cause photoinhibition and damages to the chloroplast and the photosynthetic apparatus in at least three ways. (1) The photosystem II (PSII) and the associated D1 protein are damaged at a rate exceeding the normal repair mechanism (e.g., Hill et al. (2011)) or the repair mechanisms itself is inhibited by heat stress (Takahashi et al. 2009). (2) The Calvin-Benson cycle is compromised under these conditions, through the inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the enzyme responsible for CO₂ fixation (Jones et al. 1998; Lilley et al. 2010). (3) The thylakoid membrane integrity of chloroplasts can be directly compromised by heat and high light. All of these ways are inter-related, may act in concert and result in the build-up of excess energy, ultimately leading to the generation of reactive oxygen species (ROS) (Suggett et al. 2008; Krueger et al. 2014). Overall this led to the establishment of the hypothesis that ROS and oxidative stress play a significant role in the bleaching phenomenon. Indeed, ROS produced in excess could cause major cellular damages in *Symbiodinium* and host cells. These include membrane oxidation (Tchernov et al. 2004), protein denaturation (Downs et al. 2000), damage to nucleic acids (see Lesser (2006) for review) and can trigger a cellular signaling cascade leading to cell death (Dunn et al. 2004; Richier et al. 2006; Bouchard and Yamasaki 2009) and/or the expulsion of the symbiotic algae by the host (Gates et al. 1992; Weis 2008).

Getting a better insight into the photosynthetic mechanisms in *Symbiodinium*

Phylogenetic studies conducted these last two decades have revealed that the genus *Symbiodinium* is delineated into nine lineages or clades (A-I), each comprising multiple strains or species (Pochon and Gates 2010). Different *Symbiodinium* phylotypes can exhibit a wide range of physiological responses to environmental variations and stress (e.g. Krueger et al. (2014)), thus allowing their coral hosts to occupy a wide range of environmental niches and also significantly contributing to their persistence under change (Howells et al. 2012). Therefore, the host specificities along with the external environmental conditions act as the driving forces responsible for particular pairings between both partners. Light is likely the most important of these environmental factors and recent studies showed that the vertical distribution of certain coral species could be explained by their association with symbionts adapted to the particular light regime experienced by the host. These adaptations may originate, among other, from a less sensitive PSII repair mechanism (Takahashi et al. 2009) and peculiar regulation mechanisms of the photosynthetic activity (e.g. Reynolds et al. (2008)). In the natural environment the coral holobiont has to cope with significant daily variations in light intensities that sometimes exceed *Symbiodinium* photosynthetic capacity (such as during thermal stress). This implies the existence of regulatory mechanisms that mitigate the excitation pressure when the light absorbed is in excess of that required for CO₂ assimilation. This can be

done by dissipating the energy in excess and/or diverting electrons in excess in the photosynthetic apparatus. In addition to the linear electron flow (LEF) operating during oxygenic photosynthesis, alternative electrons flows (AEF) have been widely described in higher plants and microalgae. They include cyclic electron flow around Photosystem I (PSI-CEF) and oxygen reduction by photosynthetic electrons through various processes: the Mehler reaction, chlororespiration, photorespiration, and mitochondrion-dependent reoxidation of reducing equivalents (see Cardol et al. (2011) for review). In the current context of climate changes and their impacts on symbiotic cnidarians a better understanding of the AEF present and their roles in *Symbiodinium* is needed. Indeed, despite that the existence of Mehler reaction, chlororespiration, or PSI-CEF has been strongly suggested in many reports to date, the nature and the relative amplitude of these mechanisms is still a matter of debate in *Symbiodinium*. Therefore, the purpose of the study presented in the first publication of the “scientific contribution” was to investigate the amplitude of photosynthetic alternative electron flows to oxygen (chlororespiration, Mehler reaction, mitochondrial respiration), its light dependence and the occurrence of PSI cyclic electron flow in *Symbiodinium* strains belonging to different Clades (A1, B1 and F1). Joint measurements of oxygen evolution, PSI and PSII activities allowed us to demonstrate that PSI-CEF and chlororespiratory activities are low compared to the extent of the Mehler reaction which can account for up to 50% of maximum photosynthetic electron transfer rate in all strains. This mechanism in *Symbiodinium* plays a photoprotective role. It takes place under high light intensities when the LEF to CO₂ fixation saturates, thus acting as an efficient electron sink. By alleviating the excitation pressure over PSII and PSI, it might thus prevent photoinhibition and photodamages. By acting as an efficient electron flow, it also generates an extra proton gradient across the thylakoid membranes, without net synthesis of NADPH. Thus, it might promote the synthesis of extra ATP probably required for CO₂ fixation or other cellular reactions (Cardol et al. 2011).

The dark side of the Mehler reaction

Discovered in 1951, the Mehler reaction involves the direct reduction of O₂ by PSI and leads to the production of superoxide ion (O₂^{•-}) (Mehler 1951). The generated ROS are rapidly converted into water thanks to the activities of the two chloroplast-associated enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX) (together grouped into the Mehler Ascorbate Peroxidase or MAP pathway). The flow of electrons extracted from water at the PSII level to water produced by APX, is called the water-water cycle (WWC) (Asada, 1999). The Mehler reaction is at the basis of the sink-limitation model of coral bleaching proposed by Wooldridge (2009). Indeed, the O₂ reduction by PSI and the WWC possess only a finite capacity for protection beyond which the electron transport chain becomes over-reduced, significant damage to PSII occurs and the rate of ROS production may exceeds the capacity of the cellular antioxidant network.

Although several studies have pointed out the water-water cycle as being one of the primary mediators of the ROS-mediated process leading to coral bleaching (Suggett et al. 2008; Weis 2008), the impacts of environmental stress on the O₂ reduction by PSI and the associated ROS-detoxifying enzymes remained to be determined. Based on the results of the first study presented here (Roberty et al. 2014), I analyzed the impacts of an acute thermal and light stress (conditions known to induce bleaching) on the WWC in the model *Symbiodinium* strain A1 (2nd publication). We observed that high light treatment at 26°C resulted in the up-regulation of SOD, APX and GR activities and an increased production of ROS with no significant change in the amplitude of the Mehler reaction. Under high light and at 33°C, the Mehler reaction significantly increased relative to total electron transport (75% vs 50% at 26°C). This increase was concomitant with a two-fold increase in ROS generation compared to the treatment at 26°C, while enzymes involved in the WWC were largely inactivated. These data show for the first time that combined heat and light stress inactivate antioxidant capacities of the WWC, and suggests that its photoprotective functions are overwhelmed under these conditions.

This study also indicates that cnidarians may be more prone to bleach if they harbor *Symbiodinium* cells having a highly active Mehler-type electron transport, unless they are able to quickly up-regulate their antioxidant capacities (Roberty et al. 2015). Indeed ROS accumulating in the chloroplasts, and more especially H₂O₂, will diffuse into the host tissue where damages occur and where they trigger a cellular signaling cascade that will ultimately lead to cell death (Bouchard and Yamasaki 2009) and to the phenomenon of coral bleaching (Weis 2008). The resulting loss of symbionts by the coral host will have dramatic ecological consequences on the colony and over the entire reef ecosystem (Hoegh-Guldberg 1999).

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